
UNIVERSITI PUTRA MALAYSIA

# EFFECTS OF LIQUID METABOLITE COMBINATIONS FROM *LACTOBACILLUS PLANTARUM* S ON INTESTINAL MICROFLORA, HISTOLOGY AND GROWTH PERFORMANCE OF PIGLETS

TRAN VAN THU

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By

**TRAN VAN THU**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for Degree of Master of Science**

**March 2011**

**DEDICATION**

To my farther Trần Văn Nghị and mother Trần Thị Trúc

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**EFFECTS OF LIQUID METABOLITE COMBINATIONS FROM *LACTOBACILLUS PLANTARUM* S ON INTESTINAL MICROFLORA, HISTOLOGY AND GROWTH PERFORMANCE OF PIGLETS**

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**Chairman: Associate Professor Loh Teck Chwen, PhD**

**Faculty : Agriculture**

Antibiotics has commonly used in piglets for growth promoter and diarrhoea prevention. However, excessive use of antibiotic may cause development of antibiotic resistance and residue in animal products. Application of lactic acid bacteria (LAB) has gained many beneficial effects on food preservation and animal gut health. The first experiment was conducted to select metabolite combinations produced from *Lactobacillus plantarum* by determination of pH, optical density (OD), *Lactobacillus* counts, organic acids and inhibitory activity test. The *L. plantarum* strains were TL1, RG11, RG14, RI11 and RS5. There are ten different combinations were established and each combination from 3 strains. The result of inhibitory test against *Pediococcus acidilactici* showed that Com 2 (TL1, RG11 and RI11), Com 5 (TL1, RG14 and RS5) and Com 7 (RG11, RG14 and RI11) were significantly more effective ($P < 0.05$) than the other 7 combinations. The Com 5 had the highest lactic acid concentration, whereas Com 5 and Com 7 had the highest acetic acid. Therefore, the Com 2, Com 5 and Com 7 were selected based on inhibitory activity and organic substances for

further studies. The second experiment was carried out to investigate the effects of feeding different liquid metabolite combinations produced by *L. plantarum* on growth performance, diarrhoea incidence, pH, microflora counts and volatile fatty acids (VFA) in faeces and cholesterol, fatty acids in plasma and villi height, crypt depth in intestine of piglets. A total of 120 piglets were assigned into one of five treatments: 1) -ve control (antibiotic free); 2) +ve control (clortetracycline); 3) Com 1 (metabolite of TL1, RG11 and RI11 strains); 4) Com 2 (metabolite of TL1, RG14 and RS5 strains); 5) Com 3 (metabolite of RG11, RG14 and RI11 strains). The results showed that average daily gain (ADG) and feed conversion ratio (FCR) were improved significantly ($P < 0.05$) in metabolite combination groups. However, daily feed intake (DFI) was not significantly differences ($P > 0.05$) among the treatment groups. Diarrhoea incidence and faecal pH and *Entrobacteriaceae* (ENT) were reduced when piglets fed with Com 2 metabolites as compared to -ve control. Faecal LAB counts of metabolite groups were significantly higher ($P < 0.05$) than the groups without metabolites. Total faecal VFA and duodenum villi height of Com 2 were significantly higher ($P < 0.05$) than –ve control. No significant differences ($P > 0.05$) were found in intestinal crypt depth of piglets. Additionally, feeding of metabolite Com 2 reduced cholesterol and increased essential fatty acids in plasma of piglets. The third experiment investigated the effects of different metabolite concentrations on the growth performance, diarrhoea incidence, gut environment and nutrient digestibility in piglets. A total of 40 piglets were assigned into one of five treatments: 1) -ve control (free antibiotic); 2) +ve control (0.03% antibiotic of chlortetracycline); 3) Met 1 (0.1% metabolites); 4) Met 3 (0.3% metabolites); 5) Met 5 (0.5% metabolites). The results showed the piglets fed with Met 3 and Met 5 treatments were improved significantly ($P < 0.05$) for ADG and DFI as compared to –ve control. The diarrhoea incidence of

piglets was reduced in Met 5 and +ve control groups. Moreover, feeding of 0.5% metabolites improved protein digestibility but did not improve energy utilization in piglets. There were significantly higher ($P < 0.05$) for digestial and faecal LAB counts in Met 3 and Met 5 treatments as compared to the other 3 treatments. In contrast, the pH and faecal ENT were significantly lower ($P < 0.05$) in Met 3 and Met 5 treatments. However, the highest total VFA observed in digesta was significant ($P < 0.05$) in Met 3 treatment. While, total VFA in faeces was significantly higher ($P < 0.05$) in Met 3 and Met 5 as compared to the other 3 treatments. These studies indicated that metabolite combinations produced by *L. plantarum* strains are potential alternative to antibiotics use as growth promoter in pigs. Feeding of 0.5% metabolite combination containing TL1, RG14 and RS5 strains demonstrated the best effect on the performances of piglets.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

# KESAN KOMBINASI CECAIR METABOLIK YANG DIHASILKAN DARIPADA *LACTOBACILLUS PLANTARUM* KE ATAS MIKROFLORA USUS, HISTOLOGI DAN PRESTASI TUMBESARAN KHINZIR

Oleh

**TRAN VAN THU**

**Mac 2011**

**Pengerusi : Professor Madya Loh Teck Chwen, PhD**

**Fakulti : Pertanian**

Antibiotik telah digunakan sebagai perangsang pertumbuhan dan pencegah cirit-birit bagi khinzir. Namum demikian, penggunaan antibiotik secara berlebihan mungkin mengakibatkan rintangan antibiotik dan kehadiran sisanya dalam produk haiwan. Aplikasi bakteria asid laktik (LAB) telah mendapat banyak kesan yang baik terhadap bidang pengawetan makanan dan kesihatan penghadaman haiwan. Ujikaji pertama telah dijalankan bagi memilih kombinasi metabolik yang dihasilkan daripada *Lactobacillus plantarum* dengan mengenalpasti pH, ketumpatan optik (OD), jumlah *Lactobacillus*, ujian asid organik dan aktiviti perencatan. Strain *L. plantarum* yang digunakan adalah TL1, RG11, RG14, RI11 dan RS5. Sepuluh kombinasi telah disediakan dan setiap kombinasi terdiri daripada tiga jenis strain berlainan. Keputusan ujian perencatan menunjukkan bahawa Com 2 (TL1, RG11 and RI11), Com 5 (TL1, RG14 and RS5) dan Com 7 (RG11, RG14 and RI11) adalah lebih tinggi dengan signifikan ($P < 0.05$) berbanding dengan tujuh jenis kombinasi yang lain. Kepekatan laktik asid didapati dengan signifikan ($P < 0.05$) paling tinggi pada Com 5 manakala

kepekatan paling tinggi asid asetik didapati pada Com 5 dan Com 7. Oleh yang demikian, Com 2, Com 5, dan Com 7 telah dipilih berdasarkan aktiviti perencatan terhadap *Pediococcus acidilactici* dan substrak organiknya untuk pengajian seterusnya. Ujikaji kedua telah dijalankan untuk mengkaji kesan makanan yang mengandungi cecair metabolik berlainan yang dihasilkan oleh *L. plantarum* terhadap percapaian pertumbuhan, insiden cirit-birit, pH, pengiraan mikroflora, asid lemak meruap (VFA) dalam najis dan kolesterol, asid lemak dalam plasma darah dan ketinggian villi, kedalaman krip pada usus khinzir. Sebanyak 120 khinzir telah diasingkan kepada salah satu lima rawatan berikut: 1) kawalan -ve (tiada antibiotik); 2) kawalan +ve (klorotetrasiklin); 3) Com 1 (metabolik strain TL1, RG11 dan RI11); 4) Com 2 (metabolik strain TL1, RG14 dan RS5); 5) Com 3 (metabolik strain RG11, RG14 dan RI11). Keputusan menunjukkan bahawa purata penambahan harian (ADG) dan kadar pertukaran makanan (FCR) dengan signifikan telah bertambah baik pada semua kumpulan kombinasi metabolik. Namum, pengambilan makanan harian (DFI) tidak mempunyai perbezaan signifikan ($P < 0.05$) antara semua kumpulan rawatan. Insiden cirit-birit, pH najis dan *Entrobacteriaceae* (ENT) telan berkurangan apabila khinzir diberi makanan metabolik berbanding dengan kawalan negatif. Pengiraan LAB dalam najis pada kumpulan metabolik adalah dengan signifikan lebih tinggi ($P < 0.05$) berbanding dengan kumpulan yang tiada metabolik. Jumlah VFA najis dan ketinggian vili duodenum dalam com 2 adalah signifikan lebih tinggi ($P < 0.05$) berbanding dengan kawalan negatif. Tiada perbezaan signifikan ($P < 0.05$) didapati pada kedalaman krip usus khinzir. Tambahan pula, pemberian makanan mengandungi metabolik Com 2 didapati dapat mengurangkan kolesterol dan menambah asid lemak perlu dalam plasma khinzir. Ujikaji ketiga yang dijalankan mengkaji kesan kepekatan metabolik berlainan terhadap pencapaian pertumbuhan, insiden cirit-birit, pengiraan

mikroflora dan pencernaan nutrien pada khinzir. Sebanyak 40 khinzir telah diasingkan kepada salah satu lima rawatan: 1) kawalan –ve (tiada antibiotik); 2) kawalan +ve (0.03% antibiotik klorotetrasiklin); 3) Met 1 (0.1% metabolik); 4) Met 3 (0.3% metabolik); 5) Met 5 (0.5% metabolik). Keputusan menunjukkan bahawa khinzir yang diberi makanan mengandungi rawatan Met 3 and Met 5 telah bertambah baik secara signifikan ($P < 0.05$) terhadap ADG dan DFI berbanding dengan kawalan –ve. Insiden cirit-birit pada khinzir telah berkurangan dalam rawatan Met 5 dan kawalan +ve. Tambahan pula, pemberian makanan 0.5% metabolik menambahbaik pencernaan protein tetapi tidak menambah kegunaan tenaga pada khinzir. Didapati bahawa pengiraan LAB najis dan pencernaan adalah signifikan lebih tinggi ($P < 0.05$) dalam rawatan Met 3 dan Met 5 berbanding dengan ketiga-tiga rawatan yang lain. Disebaliknya, pH dan pengiraan ENT najis adalah signifikan lebih rendah ($P < 0.05$) dalam rawatan Met 3 dan Met 5. Namum, jumlah VFA yang diperhatikan dalam digesta adalah signifikan lebih tinggi ($P < 0.05$) dalam rawatan Met 3. Sementara itu, jumlah VFA najis didapati signifikan lebih tinggi ($P < 0.05$) dalam Met 3 dan Met 5 berbanding dengan ketiga-tiga rawatan yang lain. Kajian ini memberi petunjuk bahawa kombinasi metabolik yang dihasilkan oleh strain *L. plantarum* berupaya memberi alternatif penggunaan antibiotik dalam makanan. Makanan mengandungi 0.5% kombinasi metabolik yang terdiri daripada strain TL1, RG14 and RS5 dapat menunjukkan kesan pencapaian terbaik pada khinzir.

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I certify that a Thesis Examination Committee has met on **26 November 2010** to conduct the final examination of Tran Van Thu on his Master of Science thesis entitled **"Effects of liquid metabolite from *Lactobacillus plantarum* strains on growth performance and gut health of post-weaned piglets"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the **Master of Science**.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and not concurrently, submitted for any other degree at Universiti Putra Malaysia and at any other institutions.

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**TRAN VAN THU**

Date: 23 March 2011

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